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 NEWS  3  Feb 06  Engineering Information Encompass files have new names
 NEWS  4  Feb 16  TOXLINE no longer being updated
 NEWS  5  Apr 23  Search Derwent WPINDEX by chemical structure
 NEWS  6  Apr 23  PRE-1967 REFERENCES NOW SEARCHABLE IN CAPLUS AND CA
 NEWS  7  May 07  DGENE Reload

 NEWS EXPRESS  May 23 CURRENT WINDOWS VERSION IS V6.0a,
               CURRENT MACINTOSH VERSION IS V5.0C (ENG) AND V5.0JB (JP),
               AND CURRENT DISCOVER FILE IS DATED 06 APRIL 2001
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FILE 'HOME' ENTERED AT 12:48:27 ON 31 MAY 2001

=> file medline biosis biotechno

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=> s bacteriorhodopsin or halorhodposin or or (sensory rhodopsin)

MISSING TERM 'OR OR'
The search profile that was entered contains a logical
operator followed immediately by another operator.

=> s bacteriorhodopsin or halorhodposin or (sensory rhodopsin)

L1          6561 BACTERIORHODOPSIN OR HALORHODPOSIN OR (SENSORY RHODOPSIN)

=> s l1 or phoborhodopsin

L2          6578 L1 OR PHOBORHODOPSIN

=> s l1 and hybrid?
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L3 38 L1 AND HYBRID?

=> dup rem l3

PROCESSING COMPLETED FOR L3
L4 23 DUP REM L3 (15 DUPLICATES REMOVED)

=> s l4 and chimer?

L5 1 L4 AND CHIMER?

=> d ibib abs 1-23 l4

L4 ANSWER 1 OF 23 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2001286820 MEDLINE
DOCUMENT NUMBER: 21240241 PubMed ID: 11342151
TITLE: Surface activity and film formation from the surface associated material of artificial surfactant preparations.
AUTHOR: Palmblad M; Gustafsson M; Curstedt T; Johansson J; Schurch S
CORPORATE SOURCE: Department of Clinical Chemistry, Karolinska Hospital, Stockholm, Sweden.. marie.palmbad@lab.ks.se
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (2001 Feb 9) 1510 (1-2) 106-17.
Journal code: A0W; 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010529
Last Updated on STN: 20010529
Entered PubMed: 20010508
Entered Medline: 20010524

AB Surfactant proteins B and C (SP-B and SP-C) are present in natural derived surfactant preparations used for treatment of respiratory distress syndrome. Herein the surface activity of an SP-C analogue (SP-C(LKS)), a **hybrid** peptide between SP-C and **bacteriorhodopsin** (SP-C/BR) and a model peptide (KL(4)) was studied with a captive bubble surfactometer (CBS). The peptides were mixed with either 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)/phosphatidylglycerol (PG) (7:3, by weight) or DPPC/PG/palmitic acid (68:22:9, by weight) at a concentration of 1 mg/ml in HEPES buffer, pH 6.9 and a polypeptide/lipid weight ratio of 0.02--0.03. In some lipid/peptide preparations also 2% of SP-B was included. Adsorption, monitored as surface tension vs. time for 10 min after bubble formation did not show discernible differences for the whole set of preparations. Equilibrium surface tensions of approximately 25 mN/m were reached after 5--10 min for all preparations, although those with SP-C/BR appeared not to reach end point of adsorption within 10 min. Area compression needed to reach minimum surface tension of 0.5--2.0 mN/m was least for the KL(4) preparation, about 13% in the first cycle. 3% SP-C(LKS) in DPPC:PG (7:3, by weight) reached minimum surface tension upon 27% compression in the first cycle. If DPPC:PG:PA (68:22:9, by weight) was used instead only 16% area compression was needed and 14% if also 2% SP-B was included. 3% SP-C(LKS) in DPPC:PG (7:3, by weight)+2% SP-B needed 34% compression to reach minimum surface tension. The replenishment of material from a surface associated surfactant reservoir was estimated with subphase depletion experiments. With the 2% KL(4) preparation incorporation of excess material took place at a surface tension of 25--35 mN/m during stepwise bubble expansion and excess material equivalent to 4.3 monolayers was found. When 2% SP-B was added to 3% SP-C(LKS) in

DPPC:PG (7:3, by weight) the number of excess monolayers increased from 1.5 to 3.6 and incorporation took place at 30--40 mN/m. When SP-B was added to 3% SP-C(LKS) in DPPC:PG:PA (68:22:9, by weight) the number of excess monolayers increased from 0.5 to 3.4 and incorporation took place at 40--50 mN/m. With 2% SP-C/BR incorporation took place at 40--45 mN/m, frequent instability clicks were observed and excess material of approximately 1.1 monolayer was estimated.

L4 ANSWER 2 OF 23 BIOTECHNO COPYRIGHT 2001 Elsevier Science B.V.
ACCESSION NUMBER: 1999:29536814 BIOTECHNO
TITLE: Expression, purification, and structural characterization of the **bacteriorhodopsin** -aspartyl transcarbamylase fusion protein
AUTHOR: Turner G.J.; Miercke L.J.W.; Mitra A.K.; Stroud R.M.; Betlach M.C.; Winter-Vann A.
CORPORATE SOURCE: G.J. Turner, Department of Physiology/Biophysics, Univ. of Miami School of Medicine, P.O. Box 016430, Miami, FL 33101, United States.
E-mail: gturner@chroma.med.miami.edu
SOURCE: Protein Expression and Purification, (1999), 17/2 (324-338), 72 reference(s)
CODEN: PEXPEJ ISSN: 1046-5928
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1999:29536814 BIOTECHNO
AB We are testing a strategy for creating three-dimensional crystals of integral membrane proteins which involves the addition of a large soluble domain to the membrane protein to provide crystallization contacts. As a test of this strategy we designed a fusion between the membrane protein **bacteriorhodopsin** (BR) and the catalytic subunit of aspartyl transcarbamylase from Escherichia coli. The fusion protein (designated BRAT) was initially expressed in E. coli at 51 mg/liter of culture, to yield active aspartyl transcarbamylase and an unfolded bacterio-opsin (BO) component. In Halobacterium salinarum, BRAT was expressed at a yield of 7 mg/liter of culture and formed a high-density purple membrane. The visible absorption properties of BRAT were indistinguishable from those of BR, demonstrating that the fusion with aspartyl transcarbamylase had no effect on BR structure. Electron microscopy of BRAT membrane sheets showed that the fusion protein was trimeric and organized in a two-dimensional crystalline lattice similar to that in the BR purple membrane. Following solubilization and size-exclusion purification in sodium dodecyl sulfate, the BO portion of the fusion was quantitatively refolded in tetradecyl maltoside (TDM). Ultracentrifugation demonstrated that BR and BRAT-TDM mixed micelles had molecular masses of 138 and 162 kDa, respectively, with a stoichiometry of one protein per micelle. High TDM concentrations (>20 mM) were required to maintain BRAT solubility, hindering three-dimensional crystallization trials. We have demonstrated that BR can functionally accommodate massive C-terminal fusions and that these fusions may be expressed in quantities required for structural investigation in H. salinarum.

L4 ANSWER 3 OF 23 BIOTECHNO COPYRIGHT 2001 Elsevier Science B.V.
ACCESSION NUMBER: 1998:28479774 BIOTECHNO
TITLE: Functional expression of green fluorescent protein derivatives in Halobacterium salinarum
AUTHOR: Nomura S.; Harada Y.
CORPORATE SOURCE: Y. Harada, Biomolecular Engin. Research Inst., 6-2-3 Furuedai, Suita, Osaka 565-0874, Japan.
E-mail: harada@beri.co.jp
SOURCE: FEMS Microbiology Letters, (1998), 167/2 (287-293), 23 reference(s)
CODEN: FMLED7 ISSN: 0378-1097
PUBLISHER ITEM IDENT.: S0378109798004030
DOCUMENT TYPE: Journal; Article

COUNTRY: Netherlands
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1998:28479774 BIOTECHNO
AB We investigated the applicability of the green fluorescent protein (GFP) of Aequorea victoria as a reporter for gene expression in an extremely halophilic organism: Halobacterium salinarum. Two recombinant GFPs were fused with **bacteriorhodopsin**, a typical membrane protein of H. salinarum. These fusion proteins preserved the intrinsic functions of each component, **bacteriorhodopsin** and GFP, were expressed in H. salinarum under conditions with an extremely high salt concentration, and were proved to be properly localized in its plasma membrane. These results suggest that GFP could be used as a versatile reporter of gene expression in H. salinarum for investigations of various halophilic membrane proteins, such as **sensory rhodopsin** or phoborhodopsin. Copyright (C) 1998 Federation of European Microbiological Societies.

L4 ANSWER 4 OF 23 BIOTECHNO COPYRIGHT 2001 Elsevier Science B.V.
ACCESSION NUMBER: 1998:28299116 BIOTECHNO
TITLE: Mapping of dopamine D.sub.3 receptor binding site by pharmacological characterization of mutants expressed in CHO cells with the Semliki Forest virus system
AUTHOR: Lundstrom K.; Turpin M.P.; Large C.; Robertson G.; Thomas P.; Lewell X.-Q.
CORPORATE SOURCE: K. Lundstrom, Glaxo-Wellcome Medicines Res. Centre, Stevenage, Herts SG1 2NY, United Kingdom.
SOURCE: Journal of Receptor and Signal Transduction Research, (1998), 18/2-3 (133-150), 28 reference(s)
CODEN: JRETET ISSN: 1079-9893
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1998:28299116 BIOTECHNO
AB Nine mutants and the wild-type human dopamine D.sub.3 receptor were expressed at high levels in BHK and CHO cells using the Semliki Forest virus system and were analysed for receptor binding with several structurally different dopamine D.sub.3 ligands. The mutation His349Leu showed a significant decrease in pK(i) values for raclopride, dopamine and GR218231, but an increase in affinity for GR99841. Thr369Val had an increase in pK(i) for both GR99841 and 7-OH-DPAT. The receptor modelling based on sequence alignment with **bacteriorhodopsin** indicated that Thr369 and His349 are located on the inside of the ligand binding pocket and the effect of the mutagenesis was therefore expected. The change in binding affinity for Thr369Val could be due to the location in the transmembrane domain VII close to the aspartate residue in domain III, the postulated counter ion for dopamine.

L4 ANSWER 5 OF 23 MEDLINE
ACCESSION NUMBER: 1998051971 MEDLINE
DOCUMENT NUMBER: 98051971 PubMed ID: 9390298
TITLE: Towards a **bacteriorhodopsin**-silicon neuromorphic photosensor.
AUTHOR: Martin C H; Chen Z P; Birge R R
CORPORATE SOURCE: W. M. Keck Center for Molecular Electronics, Syracuse University, NY 13244, USA.
CONTRACT NUMBER: GM-34548 (NIGMS)
SOURCE: PACIFIC SYMPOSIUM ON BIOCOMPUTING, (1997) 268-79.
Journal code: CWQ; 9711271.
PUB. COUNTRY: Singapore
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199801
ENTRY DATE: Entered STN: 19980129
Last Updated on STN: 19980129

[redacted]ered Medline: 19980115

AB We describe our [redacted]forts towards constructing a hy[redacted]d protein-silicon neuromorphic photosensor based on the photo-active protein bacteriohodopsin. This protein displays an differential photosensitivity similar to the response of the receptive field of an X-type retinal ganglion cell. Similar bacteriohodopsin photoelectrode arrays display inherent edge detection and motion enhancement. We discuss challenges associated with constructing and understanding the protein-silicon interface and possible chemical solutions for our experimental device.

L4 ANSWER 6 OF 23 BIOTECHNO COPYRIGHT 2001 Elsevier Science B.V.
ACCESSION NUMBER: 1996:26081623 BIOTECHNO
TITLE: Overexpression of bacterio-opsin in Escherichia coli as a water-soluble fusion to maltose binding protein: Efficient regeneration of the fusion protein and selective cleavage with trypsin
AUTHOR: Chen G.-Q.; Gouaux J.E.
CORPORATE SOURCE: Biochemistry/Molecular Biology Dept., University of Chicago, 920 East 58th Street,Chicago, IL 60637, United States.
SOURCE: Protein Science, (1996), 5/3 (456-467)
CODEN: PRCIEI ISSN: 0961-8368
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1996:26081623 BIOTECHNO
AB **Bacteriorhodopsin** (bR) is a light-driven proton pump from Halobacterium salinarium and is a model system for studying membrane protein folding, stability, function, and structure. bR is composed of bacterio-opsin (bO), the 248-amino acid apo protein, and all-trans retinal, which is linked to lysine 216 via a protonated Schiff base. A bO gene (sbOd) possessing 29 unique restriction sites and a carboxyl-terminal purification epitope (1D4, nine amino acids) has been designed and synthesized. Overexpression of bO was achieved by fusion to the carboxyl terminus of maltose binding protein (MBP). The expressed fusion protein (MBP-sbO-1D4) formed inclusion bodies in Escherichia coli and, following solubilization with urea and removal of the urea by dialysis, approximately 170 mg of .sim.75% pure MBP-sbO-1D4 was obtained from 1 L of culture. MBP-sbO-1D4 formed high molecular weight (>=2,000 kDa) oligomers that were water-soluble. The synthetic bO with the 1D4 tag (sbO- 1D4) was separated from MBP by trypsin cleavage at the factor Xa site between the MBP and sbO-1D4 domains. Selective trypsin cleavage at the factor Xa site, instead of at the 14 other potential trypsin sites within bO, was accomplished by optimization of the digestion conditions. Both MBP sbO-1D4 and sbO-1D4 were regenerated with all-trans retinal ánd purified to homogeneity. In general, 6-10 mg of sbR-1D4 and 52 mg of MBP-sbR-1D4 were obtained from 1 L of cell culture. No significant differences in terms of UV/vis light absorbance, light/dark adaptation, and photocycle properties were observed among sbR-1D4, MBP sbR-1D4, and bR from H. salinarium.

L4 ANSWER 7 OF 23 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 95286981 MEDLINE
DOCUMENT NUMBER: 95286981 PubMed ID: 7769231
TITLE: Use of antibody fragments (Fv) in immunocytochemistry.
AUTHOR: Kleymann G; Ostermeier C; Heitmann K; Haase W; Michel H
CORPORATE SOURCE: Max-Planck-Institut Fur Biophysik, Abteilung Molekulare Membranbiologie, Frankfurt, Germany.
SOURCE: JOURNAL OF HISTOCHEMISTRY AND CYTOCHEMISTRY, (1995 Jun) 43 (6) 607-14.
Journal code: IDZ; 9815334. ISSN: 0022-1554.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 199507
ENTRY DATE: Entered STN: 19950713
Last Updated on STN: 19950713
Entered Medline: 19950706

AB We developed a novel antibody fragment (Fv) technique for localization and determination of the surface topology of membrane protein complexes by immunogold electron microscopy. Several **hybridoma** cell lines producing murine monoclonal antibodies (MAbs) raised against bacterial membrane proteins were established. The cDNAs coding for the variable domains of the MAbs were cloned and expressed in Escherichia coli. The engineered Fv fragments served as trifunctional adapter molecules. The Fv fragment binds to the epitope of the membrane protein. The Strep tag fused to the VH chain was used for one-step affinity purification of the Fv fragments. Immunological detection of the membrane protein-bound Fv fragments in electron microscopy was accomplished either via the Strep tag with colloidal gold-labeled streptavidin or via the c-myc tag, which was fused to the VL chain, in combination with the c-myc tag-specific antibody 9E10 and a colloidal gold-labeled secondary antibody. We examined four Fv fragments directed against the cytochrome c oxidase or the ubiquinol-cytochrome c oxidoreductase of Paracoccus denitrificans and **bacteriorhodopsin** of Halobacterium halobium to show that this method is generally applicable. In all cases the Fv fragments showed the same results as their corresponding parent antibodies in electron microscopic immunostaining and other applications.

L4 ANSWER 8 OF 23 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 95251620 MEDLINE
DOCUMENT NUMBER: 95251620 PubMed ID: 7733894
TITLE: Secondary structure and biophysical activity of synthetic analogues of the pulmonary surfactant polypeptide SP-C.
AUTHOR: Johansson J; Nilsson G; Stromberg R; Robertson B; Jornvall H; Curstedt T
CORPORATE SOURCE: Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden.
SOURCE: BIOCHEMICAL JOURNAL, (1995 Apr 15) 307 (Pt 2) 535-41.
Journal code: 9YO; 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 19950608
Last Updated on STN: 19950608
Entered Medline: 19950526

AB Native pulmonary-surfactant-associated lipopolypeptide SP-C, its chemically depalmitoylated form and several synthetic analogues lacking the palmitoylcysteine residues were analysed for secondary structure in phospholipid micelles and for biophysical activity in 1,2-dipalmitoyl-sn-glycero-3- phosphocholine/phosphatidylglycerol/palmitic acid (68:22:9, by wt.). Compared with the native molecule, with the entire poly-valyl part in a known alpha-helical conformation, depalmitoylated SP-C was found to be still mainly alpha-helical, but with an approx. 20% decrease in the helical content. A synthetic **hybrid** polypeptide where the entire poly-valyl alpha-helical part of native SP-C had been replaced with the amino acid sequence of a transmembrane helix of **bacteriorhodopsin** is also predominantly alpha-helical. In contrast, synthetic SP-C analogues lacking only the palmitoyl groups, by replacement of the palmitoylcysteine residues with cysteine, phenylalanine or serine, or lacking the positively charged amino acids by replacement with alanine, are considerably less alpha-helical than both native and depalmitoylated SP-C. The data indicate that the SP-C palmitoyl groups are important for maintenance of the

alpha-helical conformation in parts of the polypeptide, and that the poly-valyl alpha-helical conformation is not fully formed in synthetic SP-C polypeptides. Furthermore, the helical structure of both native and depalmitoylated SP-C in dodecylphosphocholine micelles is very resistant to thermal denaturation, exhibiting ordered structure at 90 degrees C. The alpha-helical content grossly parallels the peptide-induced acceleration of the spreading of phospholipids at an air/water interface and the increase of surface pressure. The data suggest that the alpha-helical conformation itself, rather than just the covalent structure, is of prime importance for the biological function of synthetic pulmonary-surfactant peptides.

L4 ANSWER 9 OF 23 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 95023912 MEDLINE
DOCUMENT NUMBER: 95023912 PubMed ID: 7937771
TITLE: Photoactive mitochondria: in vivo transfer of a light-driven proton pump into the inner mitochondrial membrane of Schizosaccharomyces pombe.
AUTHOR: Hoffmann A; Hildebrandt V; Heberle J; Buldt G
CORPORATE SOURCE: Forschungszentrum Julich, Institut fur Biologische Informationsverarbeitung, Germany.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1994 Sep 27) 91 (20) 9367-71. Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199410
ENTRY DATE: Entered STN: 19941222
Last Updated on STN: 19970203
Entered Medline: 19941027

AB The light-driven proton pump **bacteriorhodopsin** (bR) from Halobacterium salinarium has been genetically transferred into the inner mitochondrial membrane (IM) of the eukaryotic cell Schizosaccharomyces pombe, where the archaebacterial proton pump replaces or increases the proton gradient usually formed by the respiratory chain. For targeting and integration, as well as for the correct orientation of bR in the IM, the bacterioopsin gene (bop) was fused to signal sequences of IM proteins. Northern and Western blot analysis proved that all **hybrid** gene constructs containing the bop gene and a mitochondrial signal sequence were expressed and processed to mature bR. Fast transient absorption spectroscopy showed photocycle activity of bR integrated in the IM by formation of the M intermediate. Experiments with the pH-sensitive fluorescence dye 2',7'-bis(2-carboxyethyl)-5 (and -6)-carboxyfluorescein revealed bR-mediated proton pumping from the mitochondrial matrix into the intermembrane space. Glucose uptake measurements under anaerobic conditions showed that yeast cells containing photoactive mitochondria need less sugar under illumination. In summary, our experiments demonstrate the functional genetic transfer of a light energy converter to a naturally nonphotoactive eukaryotic organism.

L4 ANSWER 10 OF 23 MEDLINE
ACCESSION NUMBER: 97014958 MEDLINE
DOCUMENT NUMBER: 97014958 PubMed ID: 9116168
TITLE: Molecular modelling of CCK-A receptors.
AUTHOR: Van Der Bent A; Ijzerman A P; Soudijn W
CORPORATE SOURCE: Center for Bio-Pharmaceutical Sciences, Division of Medicinal Chemistry, Leiden, The Netherlands.
SOURCE: DRUG DESIGN AND DISCOVERY, (1994 Nov) 12 (2) 129-48. Journal code: A5B; 9200627. ISSN: 1055-9612.
PUB. COUNTRY: Switzerland
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 1[redacted]704
ENTRY DATE: E[redacted]ered STN: 19970506
Last Updated on STN: 19970506
Entered Medline: 19970423

AB Recently, the primary structure of the cholecystokinin A-type (CCK-A) receptor has been determined. From the Kyte-Doolittle-predicted hydrophobic stretches of this sequence and the transmembrane domains of **bacteriorhodopsin**, a membrane-bound protein of known tertiary structure, a three-dimensional model of the membrane-embedded part of this receptor was built. Subsequently, the modelled receptor pore was searched for a binding site that matches the structural and conformational characteristics of the parent classes of the antagonists devazepide and lorglumide. In addition, the binding mode of **hybrid** analogues of these reference compounds was examined. The proposed antagonist, binding site includes regions in which hydrophobic, hydrogen-bonding and aromatic interactions stabilize the receptor-ligand complex.

L4 ANSWER 11 OF 23 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 94134061 MEDLINE
DOCUMENT NUMBER: 94134061 PubMed ID: 8302281
TITLE: Intramolecular interactions in muscarinic acetylcholine receptors studied with chimeric m2/m5 receptors.
AUTHOR: Pittel Z; Wess J
CORPORATE SOURCE: National Institute of Diabetes and Digestive and Kidney Diseases, Laboratory of Bioorganic Chemistry, Bethesda, Maryland 20892.
SOURCE: MOLECULAR PHARMACOLOGY, (1994 Jan) 45 (1) 61-4.
Journal code: NGR; 0035623. ISSN: 0026-895X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199403
ENTRY DATE: Entered STN: 19940318
Last Updated on STN: 19980206
Entered Medline: 19940310

AB Current models of the three-dimensional structures of muscarinic acetylcholine receptors and other G protein-coupled receptors are based primarily on high-resolution electron diffraction data obtained with **bacteriorhodopsin**, the molecular structure of which is characterized by the presence of seven alpha-helical transmembrane domains (TM I-VII). However, **bacteriorhodopsin** does not couple to G proteins and its primary sequence lacks a series of amino acids that are conserved among virtually all G protein-coupled receptors. Therefore, it remains to be shown experimentally whether the molecular structures of these functionally different proteins are in fact identical. To address this question, we have analyzed the pharmacological properties of a series of **hybrid** human m2/m5 muscarinic receptors. Initially, we identified several chimeric constructs that, upon transient expression in COS-7 cells, were unable to bind significant amounts of the muscarinic antagonists N-[3H]methylscopolamine and [3H]quinuclidinyl benzilate. A common structural feature of these constructs was the presence of m2 receptor sequence in TM VII and of m5 receptor sequence in TM I. The ligand-binding activity of these "pharmacologically inactive" **hybrid** receptors could be restored by replacing TM I (consisting of m5 receptor sequence) with the corresponding m2 receptor domain. These data provide the first direct experimental evidence that the molecular architecture of muscarinic receptors (and, most likely, that of other G protein-coupled receptors) resembles that of **bacteriorhodopsin**, in that the seven TM helices are arranged in a ring-like fashion such that TM I lies directly adjacent to TM VII.

L4 ANSWER 12 OF 23 BIOTECHNO COPYRIGHT 2001 Elsevier Science B.V.
ACCESSION NUMBER: 1992:22309699 BIOTECHNO
TITLE: The role of the membrane domain in the regulated

degradation of 3-hydroxy- 3-methylglutaryl coenzyme A reductase
AUTHOR: Chun K.T.; Simoni R.D.
CORPORATE SOURCE: Department of Biological Sciences, Stanford University,Stanford, CA 94305-5020, United States.
SOURCE: Journal of Biological Chemistry, (1992), 267/6 (4236-4246)
CODEN: JBCHA3 ISSN: 0021-9258
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1992:22309699 BIOTECHNO
AB We have constructed a series of mutations in the membrane and linker domains of Syrian hamster 3-hydroxy-3-methylglutaryl-(HMG) CoA reductase in order to determine the regions critical for the regulated degradation of the enzyme. In transfected Chinese hamster ovary cells, we have expressed a fusion protein, HMGal, which consists of the membrane and linker domains of the Syrian hamster HMG-CoA reductase fused to .beta.-galactosidase. Using this fusion protein, we have determined that a deletion of 64 amino acids from the central region of the membrane domain causes the protein to be degraded extremely rapidly. In addition, deletion of PEST sequences has little effect on degradation, but deletion of the linker domain makes the protein's degradation insensitive to sterols and mevalonate. In addition to deletion mutations, we have systematically replaced each hydrophobic, putative membrane spanning region of the membrane domain with the first transmembrane sequence from **bacteriorhodopsin**. Replacement of span 4 has no effect on degradation. Replacements of spans 5 or 6 result in a protein which has a normal basal rate of degradation, but this rate of degradation is not accelerated by mevalonate, low density lipoprotein, or 25-hydroxycholesterol. Replacement of span 3 results in a protein whose degradation is similarly not accelerated by sterols or mevalonte, but since this protein might be mislocalized, these results are inconclusive. Replacement of span 7 yields a short-lived protein which is degraded more rapidly in response to mevalonate but not in response to exogenous sterols. Replacement of span 8 extends both the basal and mevalonate-accelerated half-life about 5-fold. This work begins to define the critical regions for regulated degradation within the membrane domain of HMG-CoA reductase.

L4 ANSWER 13 OF 23 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 6
ACCESSION NUMBER: 1992:351785 BIOSIS
DOCUMENT NUMBER: BA94:44010
TITLE: BACTERIAL RHODOPSINS OF NEWLY ISOLATED HALOBACTERIA.
AUTHOR(S): OTOMO J; TOMIOKA H; SASABE H
CORPORATE SOURCE: FRONTIER RES. PROGRAM, RIKEN INST., WAKO, SAITAMA 351-01, JPN.
SOURCE: J GEN MICROBIOL, (1992) 138 (5), 1027-1037.
CODEN: JGMIAN. ISSN: 0022-1287.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB Several new halobacterial strains were isolated from crude solar salts commercially produced in Mexico and Australia. The presence of **bacteriorhodopsin** (BR)- and halorhodopsin (HR)-like pigment in their total membrane fraction was measured by flash spectroscopy and light-induced ion pumping activity. Two of these strains contained both BR- and HR-like pigments; the other contained only BR-like pigment. DNA **hybridization** analysis with probes from bacterioopsin and haloopsin genes revealed that the genes encoding the BR- and HR-like pigments were not homologous to those found in Halobacterium halobium R1. In addition, the presence of **sensory rhodopsin** (SR)- and phoborhodopsin (PR)-like pigment in these membranes was detected by

flash spectroscopy. The kinetics of the cyclic photoreaction of the SR-like pigment was more than 15 times slower than that of H. halobium. A PR-like pigment existed in two strains, and the kinetics of the cyclic photoreaction of the PR-like pigment was similar to that found in H. halobium.

L4 ANSWER 14 OF 23 BIOTECHNO COPYRIGHT 2001 Elsevier Science B.V.
ACCESSION NUMBER: 1990:20211901 BIOTECHNO
TITLE: An efficient system for the synthesis of **bacteriorhodopsin** in Halobacterium halobium
AUTHOR: Ni B.; Chang M.; Duschl A.; Lanyi J.; Needleman R.
CORPORATE SOURCE: Department of Biochemistry, Wayne State University School of Medicine, Detroit, MI 48201, United States.
SOURCE: Gene, (1990), 90/1 (169-172)
CODEN: GENED6 ISSN: 0378-1119
DOCUMENT TYPE: Journal; Article
COUNTRY: Netherlands
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1990:20211901 BIOTECHNO
AB The mechanism by which **bacteriorhodopsin** (BR) transports protons across the cell membrane of Halobacterium halobium is actively studied in many laboratories. Currently available systems for the synthesis of mutant proteins obtained by site-directed mutagenesis of the gene encoding BR (bop) require reconstitution of the denatured polypeptide after its synthesis Escherichia coli or yeast; this approach is technically difficult and labor intensive, and raises questions about possible differences between in vivo and in vitro folding. Using a newly described transformation system and a halobacterial plasmid vector, we show that it is possible to reintroduce the bop gene into BR.sup.- strains of H. halobium. The bop-carrying plasmid expresses native BR in amounts similar to those obtained in several wild type strains. This system allows facile site-directed mutagenesis in halophilic archaebacteria.

L4 ANSWER 15 OF 23 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 90133932 MEDLINE
DOCUMENT NUMBER: 90133932 PubMed ID: 2614846
TITLE: Tertiary structure of **bacteriorhodopsin**. Positions and orientations of helices A and B in the structural map determined by neutron diffraction.
AUTHOR: Popot J L; Engelman D M; Gurel O; Zaccai G
CORPORATE SOURCE: Institut de Biologie Physico-Chimique, Paris, France.
CONTRACT NUMBER: GM22778 (NIGMS)
GM39546 (NIGMS)
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1989 Dec 20) 210 (4) 829-47.
Journal code: J6V; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199003
ENTRY DATE: Entered STN: 19900328
Last Updated on STN: 19970203
Entered Medline: 19900314
AB Positions and rotations of two helices in the tertiary structure of **bacteriorhodopsin** have been studied by neutron diffraction using reconstituted, **hybrid** purple membrane samples. Purple membrane was biosynthetically 2H-labeled at non-exchangeable hydrogen positions of leucine and tryptophan residues. Two chymotryptic fragments were purified, encompassing either the first two or the last five of the seven putative transmembrane segments identified in the amino acid sequence of **bacteriorhodopsin**. The 2H-labeled fragments, diluted to variable extents with the identical, unlabeled fragment, were mixed with their unlabeled counterpart; **bacteriorhodopsin** was then renatured and reconstituted. The crystalline purple membrane samples thus obtained

contained **hybrid** **bacteriorhodopsin** molecules in which certain transmembrane segments had been selectively 2H-labeled to various degrees. Neutron diffraction powder patterns were recorded and analyzed both by calculating difference Fourier maps and by model building. The two analyses yielded consistent results. The first and second transmembrane segments in the sequence correspond to helices 1 and 7 of the three-dimensional structure, respectively. Rotational orientations of these two helices were identified using best fits to the observed diffraction intensities. The data also put restrictions on the position of the third transmembrane segment. These observations are discussed in the context of folding models for **bacteriorhodopsin**, the environment of the retinal Schiff base, and site-directed mutagenesis experiments.

L4 ANSWER 16 OF 23 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 87241740 MEDLINE
DOCUMENT NUMBER: 87241740 PubMed ID: 3036168
TITLE: [Expressing plasmid vectors on the basis of Escherichia coli beta-galactosidase gene fragments].
Ekspressiruiushchie plazmidnye vektory na osnove fragmentov gena beta-galaktozidazy Escherichia coli.
AUTHOR: Chakhmakhcheva O G; Mirskikh O V; Chiong N H; Efimov V A
SOURCE: BIOORGANICHESKAIA KHIMIIA, (1987 Mar) 13 (3) 350-8.
Journal code: 9Z8; 7804941. ISSN: 0132-3423.
PUB. COUNTRY: USSR
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Russian
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198707
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19900305
Entered Medline: 19870716

AB With the use of synthetic DNA fragments, a set of new plasmid vectors has been obtained. The vectors provided high level expression of peptides and small proteins in E. coli as fusions with fragments of beta-galactosidase of various length. These vectors were used to achieve expression of a synthetic gene for a functionally active fragment of **bacteriorhodopsin**. The yields of **hybrid** proteins consisting of beta-galactosidase and **bacteriorhodopsin** fragments were in the range of 5-30% from the total amount of cellular protein.

L4 ANSWER 17 OF 23 MEDLINE
ACCESSION NUMBER: 86008085 MEDLINE
DOCUMENT NUMBER: 86008085 PubMed ID: 2995317
TITLE: Restoration of bacterioopsin gene expression in a revertant of Halobacterium halobium.
AUTHOR: Pfeifer F A; Boyer H W; Betlach M C
CONTRACT NUMBER: GM28749 (NIGMS)
GM31785 (NIGMS)
SOURCE: JOURNAL OF BACTERIOLOGY, (1985 Oct) 164 (1) 414-20.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198511
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19990129
Entered Medline: 19851114

AB Restoration of bacterioopsin (bop) gene expression in a revertant of Halobacterium halobium was investigated. The phenotype of the revertant is the result of a translocation of the 588-base-pair (bp) sequence "ISH25", adjacent to an ISH24 insertion found in the parental mutant IV-4. These insertions are located about 1,400 bp upstream of the bop gene within the coding region of the putative brp (bacterioopsin-related protein) gene.

The level at which the brp gene affects bop gene expression is unknown. Analysis of bop and brp gene transcription in the wild type, mutant IV-4, and the revertant supports the hypothesis that transcription of the putative brp gene is necessary for bop gene expression in the revertant. Eight insertion mutants of the Bop revertant were analyzed to further elucidate restoration of bop gene expression in the revertant. Bop mutants of the revertant were recovered with a frequency of about 10(-4) and were found to contain insertion elements in addition to ISH24 and "ISH25". Six-eighths of these mutants have the insertion element ISH2, and two mutants have previously uncharacterized insertion elements (ISH27 [1,400 bp] and ISH28 [1,000 bp]). ISH27 and ISH28 are confined to the more A + T-rich fraction of the H. halobium genome, as are most copies of other halobacterial insertion elements. The insertion sites in the Bop mutants of the revertant mapped within the coding region of the bop gene (three mutants), immediately upstream of the bop gene presumably in the bop promoter region (two mutants), or within a region from 241 to 449 bp upstream of the bop gene (three mutants).(ABSTRACT TRUNCATED AT 250 WORDS)

L4 ANSWER 18 OF 23 MEDLINE
ACCESSION NUMBER: 85037955 MEDLINE
DOCUMENT NUMBER: 85037955 PubMed ID: 6093059
TITLE: Characterization of a halobacterial gene affecting bacterio-opsin gene expression.
AUTHOR: Betlach M; Friedman J; Boyer H W; Pfeifer F
CONTRACT NUMBER: GM31785 (NIGMS)
SOURCE: NUCLEIC ACIDS RESEARCH, (1984 Oct 25) 12 (20) 7949-59. Journal code: O8L; 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X01081
ENTRY MONTH: 198412
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19970203
Entered Medline: 19841210

AB A substantial number of spontaneous bacterio-opsin mutants of Halobacterium halobium are the result of insertion elements up to 1400 bp upstream of the bacterio-opsin (bop) gene. The nucleotide sequence of 1800 bp upstream of the bop gene has been determined. There is a 1118 bp open reading frame (ORF) located within this region which is transcribed and which coincides with the distribution of insertion elements upstream of the bop gene in Bop mutants. Therefore, we propose that there is a gene (brp gene) 526 bp upstream of the bop gene. This putative gene is transcribed in the opposite direction as the bop gene and could encode a protein of 37,500 D (359 amino acids) with a codon usage similar to bacterio-opsin. The 5' terminus of the brp transcript has been determined.

The brp transcript and the bop mRNA are complementary for 13 residues near their 5' termini and both transcripts start at or near the initiating codon of the gene. Both transcripts could form similar hairpin loop structures at their 5' termini which contain possible ribosomal binding sites. The DNA sequences immediately upstream of the bop and the brp genes have significant homologies and there is a short complementary sequence. The role of the brp gene in bacterio-opsin gene expression is unclear.

L4 ANSWER 19 OF 23 BIOTECHNO COPYRIGHT 2001 Elsevier Science B.V.
ACCESSION NUMBER: 1984:14180840 BIOTECHNO
TITLE: Bacterio-opsin mRNA in wild-type and bacterio-opsin-deficient Halobacterium halobium strains
AUTHOR: DasSarma S.; RajBhandary U.L.; Khorana H.G.
CORPORATE SOURCE: Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, United States.

SOURCE: Proceedings of the National Academy of Sciences of the
United States of America, (1984), 81/1 I (125-129)
CODEN: PNASA6
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
AN 1984:14180840 BIOTECHNO
AB We have examined transcripts corresponding to the Halobacterium halobium bacterio-opsin (BO) gene in wild-type and in BO-deficient mutant strains containing the insertion elements ISH1 and ISH2 in the BO gene. BO mRNA from the wild-type strain was purified in **hybrid** selection using single-stranded cDNA. Labeling by vaccinia virus capping enzyme and .cents..alpha.-.sup.3.sup.2P!GTP showed that it contains the 5'terminal nucleotide of the primary transcript. Sequence analysis showed that transcription begins only two nucleotides upstream of the initiator codon for BO. Two species of BO mRNA were found; the major species has a ragged 3' terminus .sim.45 nucleotides downstream from the terminator codon for BO, while the minor species is about 170 nucleotides longer at the 3' end. Analysis of the transcripts in several BO gene mutant strains by RNA gel-transfer **hybridization** showed that (i) mutants with ISH1 insertions within the NH.sub.2-terminal coding region of the gene contain no detectable transcripts, (ii) mutants with ISH2 near the middle of the coding region of the gene contain multiple incomplete transcripts, and (iii) a mutant that is partially BO deficient due to an insertion of ISH2 100 base pairs upstream of the site of initiation of transcription contains a decreased level of BO mRNA.

L4 ANSWER 20 OF 23 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1983:295851 BIOSIS
DOCUMENT NUMBER: BA76:53343
TITLE: MONO CLONAL ANTIBODIES TO RHOD OPSIN CHARACTERIZATION CROSS REACTIVITY AND APPLICATION AS STRUCTURAL PROBES.
AUTHOR(S): MOLDAY R S; MACKENZIE D
CORPORATE SOURCE: DEP. OF BIOCHEMISTRY, UNIV. OF BRITISH COLUMBIA, VANCOUVER, BRITISH COLUMBIA, CAN. V6T 1W5.
SOURCE: BIOCHEMISTRY, (1983) 22 (3), 653-660.
CODEN: BICHAW. ISSN: 0006-2960.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB Two monoclonal antibodies designated as rhodopsin (rho) 1D4 and rho 4A2 were obtained from **hybridoma** cells cloned after the fusion of mouse myeloma cells with spleen cells of a mouse immunized with bleached bovine rod outer segment disk membranes. These antibodies were specific for rhodopsin as determined by radioimmune labeling of bovine rod outer segment disk membrane proteins electrophoretically transferred from sodium dodecyl sulfate gels to CNBr[cyanogen bromide]-activated paper. Limited proteolytic digestion of rhodopsin in sealed disk membranes in conjunction with radioimmune assays indicated that the rho 1D4 antibody bound to the carboxyl-terminal segment of rhodopsin on the cytoplasmic side of disk membranes, whereas the rho 4A2 antibody bound to a determinant along the amino-terminal third of the rhodopsin polypeptide chain. Binding of the rho 4A2 antibody was sensitive to solubilization and photobleaching of rhodopsin. The rho 4A2 antibody did not bind to rhodopsin in sealed membrane disks but did bind to detergent-solubilized rhodopsin. Detergent-solubilized bleached rhodopsin was 13 times more antigenic than unbleached rhodopsin. Rhodopsin solubilized in Triton X-100 was more antigenic than rhodopsin solubilized in cholate. Apparently, the 4A2 antibody serves as a sensitive immunological probe for structural changes

of rhodopsin ca[illegible]d by solubilization and photob[illegible]ching. Both the rho 1D4 and 4A2 antibodies were also found to cross-react with frog rhodopsin but not H. halobium **bacteriorhodopsin**. The rho 4A2 antibody bound to the 3 forms of frog rhodopsin resolved by sodium dodecyl sulfate gel electrophoresis; rho 1D4 bound to only the 2 higher MW frog rhodopsins. Lectin inhibition studies using 125I-labeled succinyl-Con A [concanavalin A] and antibody inhibition showed freshly prepared bovine disks were sealed with the lectin binding sites oriented toward the inside of the disk; frozen-thawed disks were predominantly unsealed with both membrane surfaces exposed. Frog disk membrane vesicles were shown to have the same orientation.

L4 ANSWER 21 OF 23 MEDLINE
ACCESSION NUMBER: 84092752 MEDLINE
DOCUMENT NUMBER: 84092752 PubMed ID: 6318028
TITLE: Isolation of the bacterioopsin gene by colony **hybridization**.
AUTHOR: Vogelsang H; Oertel W; Oesterhelt D
SOURCE: METHODS IN ENZYMOLOGY, (1983) 97 226-41.
Journal code: MVA; 0212271. ISSN: 0076-6879.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198402
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19900319
Entered Medline: 19840214

L4 ANSWER 22 OF 23 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1982:215261 BIOSIS
DOCUMENT NUMBER: BA73:75245
TITLE: THE BACTERIO RHOD OPSIN GENE.
AUTHOR(S): DUNN R; MCCOY J; SIMSEK M; MAJUMDAR A; CHANG S H; RAJBHANDARY U L; KHORANA H G
CORPORATE SOURCE: DEP. BIOL., MASS. INST. TECHNOL., CAMBRIDGE, MASS. 02139.
SOURCE: PROC NATL ACAD SCI U S A, (1981) 78 (11), 6744-6748.
CODEN: PNASA6. ISSN: 0027-8424.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB The **bacteriorhodopsin** gene was identified in a 5.3-kilobase restriction endonuclease fragment isolated from Halobacterium halobium DNA, using a cloned c[complementary]DNA fragment as the probe. Of the 1229 nucleotides whose sequence was determined in the genomic fragment, 786 correspond to the structural gene of **bacteriorhodopsin**, 360 are upstream from the initiator methionine codon, and 83 are downstream from the COOH terminus. The **bacteriorhodopsin** gene codes for a precursor sequence of 13 amino acids at the NH2 terminus, 248 amino acids that are present in the mature protein, and an additional aspartic acid at the COOH terminus. This determination of the DNA sequence for an archaebacterial gene reveals that the standard genetic code is used; however, there is a marked preference for either G or C in the 3rd codon position. The gene does not contain any intervening sequences and no prokaryotic promoter can be identified in the region immediately upstream from the structural gene. The **bacteriorhodopsin** mRNA contains at the 5' terminus only 3 nucleotides beyond the initiating AUG codon and this terminus can form a hairpin structure. Immediately downstream from this structure there is a sequence complementary to the 3' terminus of H. halobium 16S rRNA.

L4 ANSWER 23 OF 23 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 81273067 MEDLINE
DOCUMENT NUMBER: 81273067 PubMed ID: 6943548
TITLE: **Bacteriorhodopsin**: partial sequence of mRNA provides amino acid sequence in the precursor region.
AUTHOR: Chang S H; Majumdar A; Dunn R; Makabe O; RajBhandary U L;

rana H G; Ohtsuka E; Tanaka T; iyama Y O; Ikehara M

CONTRACT NUMBER: [redacted]1479 (NIAID)
GM28289 (NIGMS)

SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1981 Jun) 78 (6) 3398-402.
Journal code: PV3; 7505876. ISSN: 0027-8424.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-J01727

ENTRY MONTH: 198110

ENTRY DATE: Entered STN: 19900316
Last Updated on STN: 19970203
Entered Medline: 19811029

AB mRNA for **bacteriorhodopsin** from Halobacterium halobium has been partially purified. By using this mRNA as template in the presence of reverse transcriptase RNA-dependent DNA nucleotidyltransferase and a 5'-[32P] synthetic oligodeoxyribonucleotide corresponding to amino acids 9-12 of **bacteriorhodopsin** as primer, we have isolated the major 5'-[32P]cDNA product, approximately 80 nucleotides long, and determined its sequence. Based on the cDNA sequence, the 5'-proximal sequence of **bacteriorhodopsin** mRNA is G-C-A-U-G-U-U-G-G-A-G-U-U-A-U-U-G-C-C-A-A-C-A-G-C-A-G-U-G-G-A-G-G-G-G-G-G-U-A-U-C
-G-C-A-G-G-C-C-C-C-A-G-A-U-C-A-C-C-C-G-G-A-C-G-U-C-C-G. This includes the expected sequence for amino acids 1-8 and shows that **bacteriorhodopsin** is synthesized as a precursor that is at least 13 amino acids longer (Met-Leu-Glu-Leu-Leu-Pro-Thr-Ala-Val-Glu-Gly-Val-Ser) at the NH2 terminus. Agarose/urea gel electrophoresis of the partially purified mRNA showed several bands; of these, a major one **hybridized** with 5'-[32P]cDNA. These results suggest that the **bacteriorhodopsin** mRNA in the partially purified preparation is homogeneous in size and that it constitutes a substantial portion of the RNA preparation subjected to electrophoresis.

=> s bacterio-opsin

L6 247 BACTERIO-OPSIN

=> s l6 not l2

L7 70 L6 NOT L2

=> s l7 and (hybrid or chimer? or fusion or fused or loop)

L8 3 L7 AND (HYBRID OR CHIMER? OR FUSION OR FUSED OR LOOP)

=> dup rem l8

PROCESSING COMPLETED FOR L8
L9 2 DUP REM L8 (1 DUPLICATE REMOVED)

=> d ibib abs 1-2

L9 ANSWER 1 OF 2 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1
ACCESSION NUMBER: 1993:232979 BIOSIS
DOCUMENT NUMBER: PREV199395124154
TITLE: Homologous overexpression of a light-driven anion pump in an archaebacterium.
AUTHOR(S): Heymann, Juergen A. W.; Havelka, Wendy A.; Oesterhelt, Dieter (1)
CORPORATE SOURCE: (1) Max-Planck-Inst. fuer Biochemie, D-8033 Martinsried Germany
SOURCE: Molecular Microbiology, (1993) Vol. 7, No. 4, pp. 623-630.

ISSN: 0950-382X.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The retinal protein halorhodopsin (HR), a light-driven chloride pump from Halobacterium halobium, was homologously overexpressed in this archaebacterium. Two DNA expression system differing in their promoter region were investigated. The haloopsin, hop, promoter coupled to the hop gene gave an increased level of HR synthesis. However, the extent of expression was driven by the copy number of the shuttle vector and did not reach the magnitude of the **bacterio-opsin**, bop, promoter system. Employing a gene **fusion** approach, the promoter for the bop gene was used to drive expression of the hope gene. A shuttle vector containing a bop-hop-cartridge was transformed into a HR-deficient strain and blueish-coloured transformants were obtained. The bop promoter expressed HR to an extent where a specific membrane fraction resembled the crystalline purple membrane of BR in terms of the lipid to protein ratio. HR could, therefore, be easily isolated in a natural membrane-bound state. This allows for direct use in biophysical studies without the application of detergents. This was the first successful overexpression of a 7-helical transmembrane protein and may be extended to other proteins of this family.

L9 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1984:266374 BIOSIS
DOCUMENT NUMBER: BA78:2854
TITLE: **BACTERIO OPSIN** MESSENGER RNA IN WILD TYPE AND **BACTERIO OPSIN** DEFICIENT HALOBACTERIUM-HALOBIUM STRAINS.
AUTHOR(S): DASSARMA S; KHORANA H G; RAJBHANDARY U L
CORPORATE SOURCE: DEP. BIOL., MASS. INST. TECHNOL., CAMBRIDGE, MA 02139, USA.
SOURCE: PROC NATL ACAD SCI U S A, (1984) 81 (1), 125-129.
CODEN: PNASA6. ISSN: 0027-8424.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB Transcripts corresponding to the H. halobium **bacterio-opsin** (BO) gene in wild-type and in BO-deficient mutant strains containing the insertion elements ISH1 or ISH2 in the BO gene were examined. BO mRNA from the wild-type strain was purified by **hybrid** selection using single-stranded c[complementary]DNA. Labeling by vaccinia virus capping enzyme and [.alpha.-32P]GTP showed that it contains the 5'-terminal nucleotide of the primary transcript. Sequence analysis showed that transcripton begins only 2 nucleotides upstream of the initiator codon for BO. Two species of BO mRNA were found; the major species has a ragged 3' terminus .simeq. 45 nucleotides downstream from the terminator codon for BO, while the minor species is about 170 nucleotides longer at the 3' end. Analysis of the transcripts in several BO gene mutant strains by RNA gel-transfer hybridization showed that mutants with ISH1 insertions within the NH2-terminal coding region of the gene contain no detectable transcripts, mutants with ISH2 near the middle of the coding region of the gene contain multiple incomplete transcripts and a mutant that is partially BO deficient due to an insertion of ISH2 100 base pairs upstream of the site of initiation of transcription contains a decreased level of BO mRNA.

=> log y

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